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BIOMIMETIC HYDROXYAPATITE FORMATION ON TITANIUM SURFACE IN SIMULATED BODY FLUIDS OF DIFFERENT CHEMICAL COMPOSITION

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A method has been developed of biomimetic hydroxyapatite (HA) biocompatible functional layer synthesis on the surface of the models of titanium articles of biomedical applications. Carboxyl-modified titanium surface acts as active nucleation site in biomineralization processes of HA synthesis which occurs in simulated body fluids (SBF) of different composition. A comparative study has been conducted on the influence of thermal conditions on the formation of HA in SBF of different chemical composition. The formation of hydroxyapatite coating on the surface of titanium has been confirmed by FTIR spectroscopy and XRD.

***Keywords**: biomimetic hydroxyapatite, titanium plates, surface modification, simulated body fluid*

INTRODUCTION

Nowadays biomedical research aims for the increase in surface biocompatibility of titanium orthopedic or dental implants by coating with biologically active materials. Titanium stands for the metallic material of choice in reconstructive medicine due to its excellent mechanical properties in bulk, relative to the low mass density, and high corrosion resistance [1].

A coating of hydroxyapatite (HA) layer can be deposited on to the metal alloy to assist the osseointegration of these implants with surrounding tissues [2]. The main reason of using HA coating on metallic substrates is to keep the mechanical properties of the metal such as load-bearing capability and, at the same time, to take advantage of the coating chemical similarity and biocompatibility with the bone [2].

Various techniques have been developed for producing HA coatings on implant surfaces, such as plasma spraying, hot isostatic pressing, sol-gel and precipitation technique. Unfortunately, HA coating of high crystallinity, which is desirable for optimum biocompatibility, could not be achieved through these methods [3].

To simulate the natural properties of HA and its formation on titanium layer, at last decade biomimetic method is commonly used, which consists in the creation of nucleation sites on the metal surface by modifying its surface by functional groups and further the process of mineralization in the simulated body fluids (SBF) similar to human plasma.

Currently a simple and potentially effective method was developed for biomimetic synthesis of nanostructured materials by self-organization of polymers and / or inorganic nanosized particles on the surface of the substrate [4]. For instance, fabrication and characterization of biomimetic collagen–apatite scaffolds with tunable structures for bone tissue engineering has been reported [6]. Authors [7] investigated the effect of collagen and vitamins on biomimetic hydroxyapatite formation. Self-organization, multi-functionality, hierarchical structural organization and self-assembly – the basic principles of biomineral structures construction, also used in biomimetics [5].

In vitro mineralization/crystallization of HA from SBF can be induced by the especially treated metal implant surfaces. Therefore, great attention has been paid to give the metal substrates a capability to induce HA formation by attaching functional groups onto their surfaces [8]. It was shown that the process of HA formation occurred on negatively charged surfaces that contained, for instance, -OH or -COOH groups [9].

Crystal formation in the aqueous solution depends on many factors including the concentration of ions, temperature, degree of saturation, and the interfacial energy. The aim of this work is comparative studies on influence of temperature and duration of immersing in different surrounding media (Tas-SBF and 10x-SBF) on biomimetic synthesis of hydroxyapatite.

EXPERIMENTAL SECTION

Experimental details. In our investigation, we used two types of SBF, with different chemical composition: Tas-SBF (titled in the name of the author) and 10x-SBF (10x concentrated simulated body fluid). Solutions were prepared according to [10]. Chemical composition of simulated body fluids are shown in Table 1.

Table 1. Composition of human blood plasma, simulated body fluid (SBF) and Tas-SBF

Ion	Concentration, mM		
	Human body plasma	SBF	Tas-SBF
Na^+	142.0	142.0	142.0
K^+	5.0	5.0	5.0
Mg^{2+}	1.5	1.5	1.5
Ca^{2+}	2.5	2.5	2.5
Cl^-	103.0	148.8	125.0
HCO^{3-}	27.0	4.2	27.0
HPO_4^{2-}	1.0	1.0	1.0
SO_4^{2-}	0.5	0.5	0.5

Procedure for the synthesis of hydroxyapatite layer on the titanium surface consists of the following stages:

(1) pre-treatment of titanium samples (cleaning, oxidation);

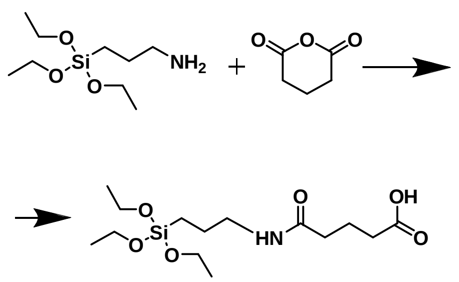
(2) preparation of the reactive functional groups on titanium surface by chemical modification;

(3) preparation of simulated body fluid medium, a chemical analogue of human plasma, which is responsible for formation of hydroxyapatite;

(4) formation of a layer of hydroxyapatite on titanium because of interaction of its functionalized surface with the surrounding medium.

For the studies, we used chemically pure titanium strip. Pre-treatment of models of implants - titanium plates was performed as follows. The surface of samples was hydroxylated with the reaction mixture of H_2O_2 (30 %) : H_2SO_4 (conc.) = 1 : 1 (by volume) with stirring for 10–15 min at room temperature. After preparing, the plates were washed with distilled water.

Synthesis of the reactive carboxyl groups on the titanium surface (sample 3) was performed by modifying triethoxysilylpropyl-carbamoyl butanoic acid (TESPCBA), obtained by reacting γ-aminopropyltriethoxysilane with glutaric anhydride. The reaction yielded TESPCBA:



Modification was carried out by keeping the titanium plates in TESPCBA solution in DMF for 24 h at room temperature (Fig. 1). Samples were washed with ethanol and distilled water.

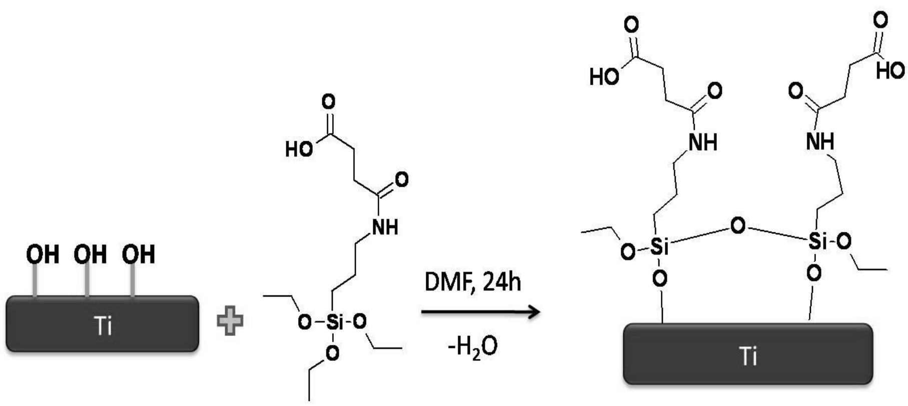


Fig. 1. Surface modification of titanium by -COOH functional groups

It has been shown that the modification of titanium surface by -COOH groups forms a layer of HA with the morphological characteristics, Ca/P ratio, and the degree of crystallinity close to those of natural hydroxyapatite [11].

We studied formation of HA within a few days at room temperature and under heating conditions (80 °C) in two types of SBF (10x-SBF and Tas-SBF).

Experimental set up. Studying of the surface of samples was performed by Fourier transform infrared spectroscopy (FTIR) (Perkin Elmer, model 1720H).

X-ray diffraction analysis (XRD) was performed using a DRON-UM1 diffractometer (Burevestnik, St. Petersburg) with CoK_α (λ = 0.17902 nm) radiation and graphite monochromator in reflected beam. XRD patterns of the samples were recorded over 2θ = 10–80° range.

RESULTS AND DISCUSSION

Obtaining HA-coating in 10x-SBF. In case of 10x-SBF, HA layer grows during no more than 24 h. After that period of time HA is clearly identifying in IR- and XRD-spectra. FTIR spectra for Ti samples immersed for 24 h in 10x-SBF are shown in Fig. 2. The absorption bands (AB) at the 1000–1100 cm^{-1} indicate the stretching vibrations of PO_4^{3-}, AB at 870–880 cm^{-1} – bending vibrations of phosphate groups, and the AB 560 and 602 cm^{-1} – bending vibrations of the PO_4^{3-}–tetrahedra. Presence of small peak at 875 cm^{-1} is characteristic for Ca-deficient apatite (identifies HA with deficient of calcium and nonstoichiometric structure).

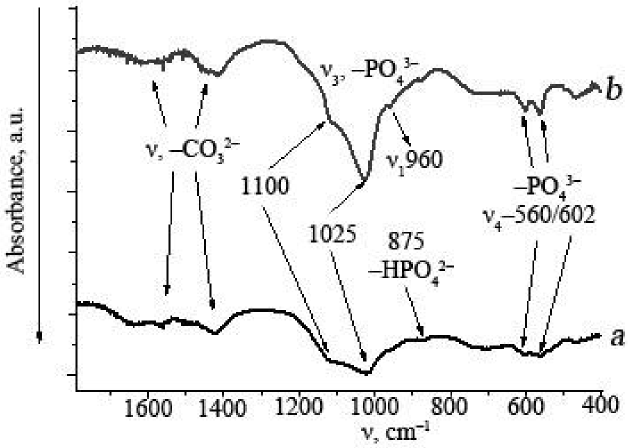


Fig. 2. FTIR spectra for Ti-samples immersed in 10x-SBF during 24 h (*a*), and for 1 h at 80 °C (*b*)

Heating of titanium samples in 10x-SBF solution leads to precipitation, what indicates the acceleration of the process of forming HA. Investigations [12] show that the HA crystallization can be measured by splitting characteristic peak at 560/602 cm^{-1} and 1070/1150 cm^{-1}. Authors assume that the sharpness of the bands at 602 and 560 cm^{-1} also shows the crystallinity of the HA. Sharpness and shape of the absorption bands for Ti samples heated in 10x-SBF indicate well crystallinity of the HA coating. Confirmation of crystallinity of the HA coating we can see from XRD (Fig. 3).

After heating in the 10x-SBF solution, the diffraction peaks ascribed to HA can be observed. XRD shows presence of a crystalline HA phase on Ti substrates. Diffraction peaks of HA are observed at 2θ = 13.56, 30.31 and 37.58 degrees.

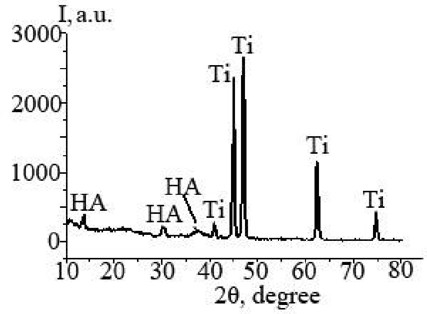


Fig. 3. XRD patterns for Ti-HA samples obtained in 10x-SBF (80 °C)

According to the data obtained, it can be concluded that formation of HA layer in 10x-SBF solution can be accelerated by elevated temperature. The effect of heat treating on the possibility to identify HA on Ti surface by physicochemical analysis presented in Table 2.

Table 2. The effect of heat treating on the possibility to identify HA on Ti surface

10x-SBF	Heating 1 h, 80 °C		RT, 24 h	
	XRD	IR	XRD	IR
possibility to identify HA	+	+	–	+

Obtaining HA-coating in Tas-SBF. In case of Tas-SBF, formation of HA takes at least 2 weeks. Fig. 4 illustrates XRD patterns of Ti-HA samples obtained after Ti immersion in Tas-SBF for 2 weeks. After 14 days of immersion, weak and broadened diffraction peaks can be found ascribed to HA. This implies that a very poor crystalline or even amorphous calcium phosphate layer on Ti substrate is formed. Diffraction peaks of HA are observed at $2\theta = 12.56$, 30.12 and 37 degrees.

FTIR spectra for Ti samples immersed in Tas-SBF are shown in Fig. 5. All absorption bands match those for apatite.

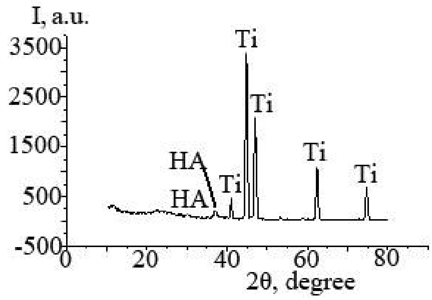


Fig. 4. XRD patterns for Ti-HA samples obtained in Tas-SBF

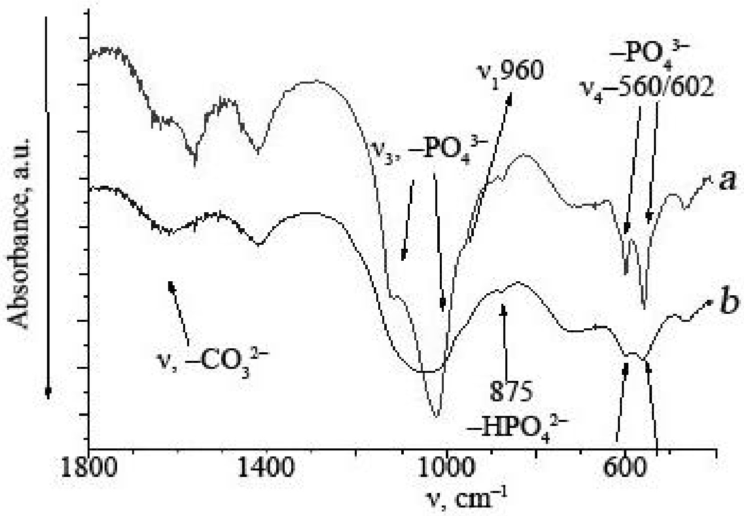


Fig. 5. FTIR of samples: Ti-HA samples immersed in Tas-SBF during 14 days (*a*), and after 1 h of heating at 80 °C (*b*)

Sharpness of the bands at 602 and 560 cm^{-1}, that indicate the crystallinity of the HA, is different for samples obtained with or without heating. Thus, the most the sharp peaks appear for the sample obtained by the 2-week immersing in Tas-SBF. This implies that crystalline layer of HA is formed on Ti substrate. On the other hand, heating of Tas-SBF solution to 80 °C do not cause layer formation, sufficient for determining the HA by XRD-method.

We can suggest that HA growth in Tas-SBF solution cannot be accelerated by elevated temperature because of low concentration of the solution unlike that in 10x-SBF.

Influence of temperature on the possibility to identify HA on Ti surface by XRD and FTIR is presented in Table 3.

Table 3. The effect of temperature conditions on the possibility to identify HA on Ti surface

Tas-SBF	Heating 1 h, 80 °C		RT, 14 days	
	XRD	IR	XRD	IR
possibility to identify HA	–	+	+	+

The Table data indicate that in case of impossibility to identify HA by XRD analysis, it is possible to consider that the crystallite size is not more than 2 nm.

CONCLUSIONS

The results obtained lead to the conclusion that formation of HA in 10x-SBF solution can be accelerated by elevated temperature. Unlike that of 10x-SBF, heating of Tas-SBF does not cause a positive effect on acceleration of the HA formation.

Fourier transform infrared spectroscopy and XRD confirmed the formation of the biomimetic hydroxyapatite coating on the surface of titanium plates with different quantity of HA as dependent on the chemical composition of simulated body fluid and on thermal conditions.

The results presented may be used to optimize the preparation of biocompatible coatings on titanium and other surfaces.

Біоміметичне формування шару гідроксоапатиту на поверхні титану в модельних фізіологічних рідинах різного хімічного складу

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Розроблено методику синтезу біосумісного функціонального шару біоміметичного гідроксоапатиту (ГА) на поверхні моделей титанових виробів медико-біологічного призначення. Синтез здійснено з середовища модельних фізіологічних рідин (МФР) різного складу шляхом біомінералізації на поверхні титану, модифікованій карбоксильними групами як активними центрами зародкоутворення. Проведено порівняльне дослідження впливу температурних умов на формування ГА в МФР різного хімічного складу. Методами ІЧ-фур'є спектроскопії та РФА підтверджено утворення покриття гідроксоапатиту на поверхні титанових зразків.

***Ключові слова**: біоміметичний гідроксоапатит, титанові пластини, модифікація поверхні, модельна фізіологічна рідина*

Биомиметическое формирование слоя гидроксоапатита на поверхности титана в модельных физиологических жидкостях различного химического состава

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Разработана методика синтеза биосовместимого функционального слоя биомиметического гидроксиапатита (ГА) на поверхности моделей титановых изделий медико-биологического назначения. Синтез осуществлен из сред модельных физиологических жидкостей (МФР) различного состава путем биоминерализации на поверхности титана, модифицированной карбоксильными группами, как активными центрами зародышеобразования. Проведено сравнительные исследования влияния температурных условий на формирование ГА в МФР различного химического состава. Методами ИК-фурье спектроскопии и РФА подтверждено образование покрытия гидроксиапатита на поверхности титановых образцов.

***Ключевые слова**: биомиметический гидроксиапатит, титановые пластины, модификация поверхности, модельная физиологическая жидкость*

REFERENCES

1. *Visan G., D., Stefan N., Duta L. et al.* Biomimetic nanocrystalline apatite coatings synthesized by Matrix Assisted Pulsed Laser Evaporation for medical applications, Mater. Sci. and Eng.: B 181 (2014) 56. doi:10.1016/j.mseb.2013.11.007.
2. *Mohseni E., Zalnezhad E., Bushroa R.* Comparative investigation on the adhesion of hydroxyapatite coating on Ti–6Al–4V implant: A review paper, Int. J. Adhes. Adhes. 48 (2014) 238. doi:10.1016/j.ijadhadh.2013.09.030.
3. *Fratzl P.* Biomimetic materials research: what can we really learn from nature's structural materials, J. R. Soc. Interface, 4 (2007) 637.
4. *Schönhoff M.* Self-assembled polyelectrolyte multilayer's, Curr. Opin. Colloid Interface Sci., 8 (2003) 86.
5. *Hirata I., Akamatsu M., Fujii E. et al.* Chemical analyses of hydroxyapatite formation on SAM surfaces modified with COOH, NH_2, CH_3, and OH functions, Dent. Mater. J., 29(4) (2010) 438. doi:10.4012/dmj.2010-017.
6. *Zengmin Xia, Xiaohua Yu, Xi Jiang et al.* Fabrication and characterization of biomimetic collagen–apatite scaffolds with tunable structures for bone tissue engineering, Acta Biomaterialia, 9 (2013) 7308.
7. *Ciobanu G., Ciobanu O.* Investigation on the effect of collagen and vitamins on biomimetic hydroxyapatite coating formation on titanium surfaces, Mater. Sci. Eng., C. 33 (2013) 1683.
8. *Dinçer M., Teker D., Sağ C.P., Öztürk K.* Enhanced bonding of biomimetic apatite coatings on surface-modified titanium substrates by hydrothermal pretreatment, Surf. Coat. Tech., 226 (2013) 27. doi:10.1016/j.surfcoat.2013.03.032.
9. *Meng-Hui Chi, Hsi-Kai Tsou, Chi-Jen Chung, Ju-Liang He.* Biomimetic hydroxyapatite grown on biomedical polymer coated with titanium dioxide interlayer to assist osteocompatible performance, Thin Solid Films., 549 (2013) 98. doi: 10.1016/j.tsf.2013.06.063.
10. *Jalota S., Bhaduri S. B., Tas A. C.* Effect of carbonate content and buffer type on calcium phosphate formation in SBF solutions, J. Mater. Sci: Mater. Med., 17 (2006) 697. doi: 10.1007/s10856-006-9680-1.
11. *Petranovska A.L., Turelyk M.P., Pylypchuk Ie.V. et al.* Biomimetic formation of hydroxyapatite on titanium surface, Metallofiz. Noveishie Tekhnol., 11(35) (2013) 1567.
12. *Tadic D., Peters F., Epple M.* Continuous synthesis of amorphous carbonated apatite, Biomaterials, 23 (2003) 2553.

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